

# Short-Period Influence of Chronic Morphine Exposure on Serum Levels of Sexual Hormones and Spermatogenesis in Rats

Hasan Ahmadnia,<sup>1</sup> Alireza Akhavan Rezayat,<sup>2,\*</sup> Mahmood Hoseyni,<sup>3</sup> Nooriye Sharifi,<sup>4</sup> Mohhamad Khajedalooee,<sup>5</sup> and Arash Akhavan Rezayat<sup>6</sup>

<sup>1</sup>Professor of Urology, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>2</sup>Assistant Professor of Urology, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>3</sup>Associated Professor of Physiology, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>4</sup>Assistant Professor of Pathology, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>5</sup>Associated Professor of Social Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran

<sup>6</sup>Member of Student Research Committee, Mashhad University of Medical Sciences, Mashhad, IR Iran

\*Corresponding author: Alireza Akhavan Rezayat, Ghaem Hospital, Ahmad Abad Ave., Mashhad, IR Iran. Tel: +98-9153148223, Fax: +98-5138022857, E-mail: alirezaakhavan30@yahoo.com

Received 2016 March 28; Revised 2016 May 30; Accepted 2016 June 05.

## Abstract

**Background:** Increased rates of addiction and its broad societal complications are well known. One of the most important systems that may malfunction in drug abusers is the reproductive system, and evaluating patients for this potential risk may lead to increased awareness.

**Materials and Methods:** Thirty 60-day-old male rats were divided into control and target groups. The target group underwent 5 mg/kg intraperitoneal injections of morphine twice a day while the control group underwent normal saline injections (at the same dosage). After 60 days, the rats were anesthetized, and after blood sampling, they underwent bilateral orchiepididymectomy. Histological and hormonal evaluations were performed on the samples.

**Results:** Levels of sex hormonal features and spermatogenesis were significantly reduced in the target group compared to the control group. LH levels showed a meaningful decrease in the target group, but FSH and testosterone levels did not. On histological section analysis, mature sperm were meaningfully decreased in the target group.

**Conclusions:** Chronic use of opioids may lead to alterations in sexual features and sexual hormones. Therefore, opioids have the potential to cause infertility. These changes may result from the effect of the drugs on the hypophysis or hypothalamus, the direct effect of the drugs on the seminiferous tubules, or a combination of both. The findings suggest that public awareness about addiction may cause decreased infertility rates.

**Keywords:** Rat, Morphine, Spermatogenesis, LH, FSH, Testosterone

## 1. Background

Drug abuse is rising dramatically, with catastrophic consequences (1). In addition to mental and physical dependence, drugs also affect physical parameters, with changes that may continue even after stopping the drugs. The genitourinary system can be affected by drugs, and due to its role in continuing the human race, research about the effects of drugs on this system is of extreme importance. Some studies have indicated that oral opium can decrease luteinizing hormone (LH), dihydrotestosterone, and follicle stimulating hormone (FSH), and can cause hypogonadism in 89% of abusers (2). The prevalence of erectile dysfunction and decreased libido is also significantly higher in drug abusers (3).

Recent investigations have shown that the abuse of morphine and other narcotics significantly decreases testosterone levels (4). It is also known that chronic mor-

phine consumption affects LH, testosterone, and body weight in rats, while no considerable changes have been observed in FSH and testicular weight (5). These findings are also supported by Cicero et al.; however, they reported a decrease in testicular and seminal vesicle weight (6). The influence of morphine on hypothalamic monoamine levels has been well-established in rats (5, 7). Gabriel et al. evaluated the effect of morphine and testosterone on rats, and concluded that morphine and testosterone together suppress the gonadotropin-releasing hormone (GnRH) (7).

The aim of this study was to determine the various effects of drugs on the genital system of rats, in order to provide a basis for human experiments. We stimulated the effects of morphine on hormonal (LH, FSH, and testosterone) and histological parameters of rat testicles.

## 2. Objectives

The aim of this study was to determine the short-period influence of chronic drug abuse on sex hormones and spermatogenesis in rats.

## 3. Materials and Methods

This was an interventional study. Thirty adult male rats (60 days old) were consecutively divided into two groups of 15 rats each, using a simple randomized method. After weighing, the case group was subjected to injections of 5 mg/kg of peritoneal morphine every 12 hours with an insulin syringe. Normal saline was injected in the control group under similar conditions. To provide the same psychological conditions, both groups were injected simultaneously. According to previous studies, the injections were performed for 60 days, approximately twice the duration of spermatogenesis (6).

### 3.1. Sampling Procedure

After 60 days, each rat was weighed. Under general anesthesia with ether, blood samples were taken from the carotid artery and collected in dry tubes. Serum was collected and used for hormonal analysis (FSH, LH, and testosterone). The rats then underwent orchietomy. A ventral midline incision was made to access the testes, then the epididymis was removed and the terminal parts were clamped. The kidneys, ureters, and ventral prostate were also detached. The testicles and seminal vesicles were separated and weighed for each group, and the samples were sent to a pathologist for review.

### 3.2. Pathological Assessment

The testicles were placed in 10% formalin and sent for pathological assessment. A questionnaire was completed for each rat. The pathological variables included seminiferous tubule diameter, sperm count per high-powered field (HPF), sperm morphology, testis weight, spermatogenesis rate, sperm motility, and Sertoli and Leydig cell indices.

Sperm motility was scored using a 5-grade scale: normal motility (grade 5), fast sperm movement in a straight direction with little deviation (grade 4), slower movement compared to grade 4 (grade 3), slower movement with great deviation (grade 2), without forward movement (grade 1), and without movement (grade 0).

The spermatogenesis rate was determined by analyzing 10 microscopic samples and the rates were divided into groups: more than 50% complete spermatogenesis in tubules was classified as complete spermatogenesis, 25% - 50% spermatogenesis in tubules was classified as medium

spermatogenesis, 1% - 25% spermatogenesis in tubules was classified as poor spermatogenesis, and < 1% spermatogenesis in tubules was considered no spermatogenesis.

Sertoli and Leydig cell indices were determined on the basis of cell counts of each kind, in 10 microscopic samples. More than 50 cells was considered normal, 10 - 50 cells was considered normal, and 5 - 10 cells was considered low.

LH, FSH, and testosterone were measured using human kits or RIA methods. Finally, data were collected for statistical analysis.

### 3.3. Statistical Analysis

Data from the biochemical and histopathological findings were analyzed with SPSS 11.5. Descriptive data were explained using measures of dispersion and the frequency distribution. For comparing serum hormone levels and weight changes, t-tests were used. The Mann-Whitney test was applied for qualitative rating. Morphology was assessed using the chi-square test.  $P < 0.05$  was considered significant.

## 4. Results

The mean weight of the control group was significantly higher than in the case group ( $P = 0.013$ ). Mean seminiferous tubule diameters were not statistically different between the two groups ( $P = 0.094$ ). Sperm counts were defined as the number of sperm per HPF. The mean sperm count was more than three times higher in the control group ( $P < 0.001$ ). LH serum levels were significantly different between the two groups ( $P = 0.002$ ), while FSH and testosterone serum levels showed no significant differences ( $P = 0.966$  and  $P = 0.263$ , respectively). Also, no significant differences were observed in testis weight ( $P = 0.176$ ). These results are shown in [Table 1](#).

Spermatogenesis was significantly decreased in the case group ( $P < 0.001$ ) ([Figures 1 and 2](#)). Sperm morphology was not significantly changed in the rats after using morphine, compared to the control group. Similarly, the Sertoli and Leydig cell indices were not significantly different between the two groups ([Figures 3 and 4](#)). Sperm motility appeared to be dramatically decreased in the case group ( $P < 0.001$ ).

## 5. Discussion

In this study, we investigated the association between drug abuse and sexual function in an animal model, as well the possible impact of chronic morphine exposure on spermatogenesis and sexual hormone levels.

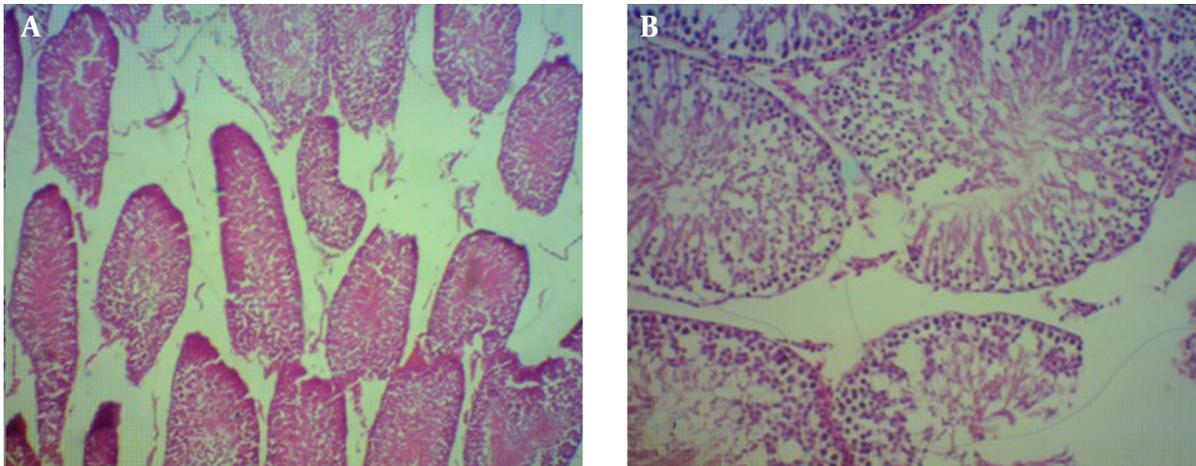


Figure 1. A, Normal view of seminiferous tubules in the control group; B, hyaline degeneration of seminiferous tubules in the morphine group

Table 1. Serum LH levels were significantly different between the case and control groups, but there were no differences in serum FSH and testosterone levels. There were no differences in mean weight or seminiferous tubule diameter between the two groups.

Parameter	Case Group	Control Group	P Value
Mean weight, g	134	157	0.013
Mean seminiferous tubule diameter, mm	135	165	0.094
Mean serum LH level, mg/dl	3.41	3.79	0.002
Mean serum FSH level, mg/dl	7.05	7.06	0.966
Mean sperm count per HPF	1.2	4.5	0.001
Mean testosterone serum level	2.34	3.1	0.263
Mean testis weight	3.06	2.87	0.176

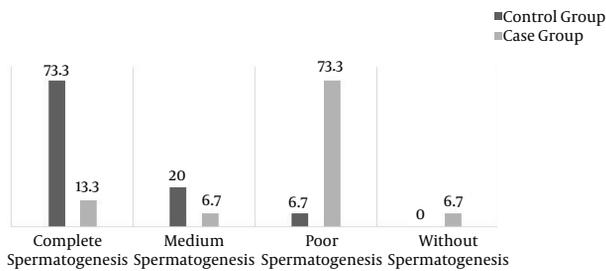


Figure 2. Comparison of Spermatogenesis Between Morphine-Treated Rats and Control Group

Maternal opioid use has significant effects on fetal outcomes, and offspring neurodevelopment has been an area of interest for decades, with studies of the effects of opium

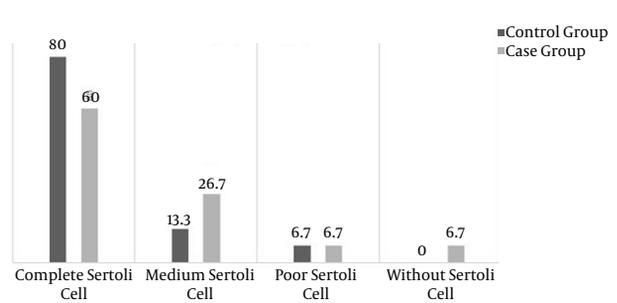


Figure 3. Distribution of Sertoli Cells in the Case and Control Groups (in Percent)

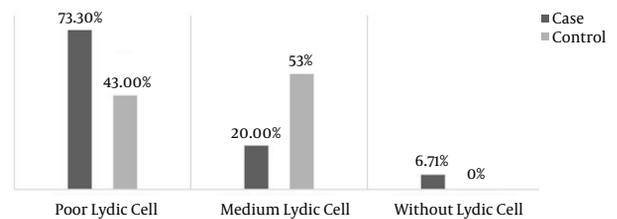


Figure 4. Distribution of Leydig Cells in the Control and Case Groups (in Percent)

use by mothers on the health and the neurodevelopment of the fetus (8, 9). The use of morphine during the teenage years has also been studied by many researchers. By using morphine in male rats during adolescence, scientists have identified important changes in sexual maturation. When these males were mated with untreated females several weeks after morphine exposure, they produced smaller litters, and parameters such as LH, sex adrenal-specific changes, and hypothalamic  $\beta$ -endorphins have

shown significant changes in the offspring of male rats that have been given morphine (6). In the present study, we observed a significant difference in LH between the morphine-induced cases and the control rats.

In contrast to our results, in other studies, female rats received morphine during early-to-mid adolescence. The morphine was discontinued for several weeks, and then these females were mated with morphine-free males. In these studies, there were no significant differences in any postnatal parameter (i.e. litter size, weight, sex ratio); however, the offspring of opium-treated rats demonstrated significant changes in behavior and emotional and sex features (10, 11).

Luteinizing hormone releasing hormone (LHRH), which is released from the hypothalamus, causes secondary effects on the pituitary gland and the release of LH and then testosterone from the testes. Our study showed that the release of testosterone was reduced in the morphine-treated group, but whether the reason for the decreased testosterone was cellular dysfunction of the testis or due to decreased LH is unknown. Also, the level of LH in the bloodstream was decreased in the case group. We did not know whether the decreased LH was due to pituitary dysfunction or to the decreased release of LHRH in the case group. It is possible that LHRH, LH, and testosterone were all affected by the morphine (6, 12, 13).

In our study, histological examinations indicated that the mean seminiferous tubule diameter and testis weight were not significantly different between the case and control groups; however, in similar studies, testis and seminiferous sizes decreased significantly. These researchers claimed that stromal atrophy of the genital organs and testosterone function in restoring the structure of seminal vesicles was the reason for the decreased size of the genital organs due to morphine (14).

The differences between the effects of morphine and oxymorphone on female fertility are not obvious. The acute doses used in morphine studies are higher and the route of administration is different than that used for oxymorphone. Despite the effects of morphine on the reproductive system and fertility of male rats, some studies showed that oxymorphone did not affect these parameters. In another study, oxymorphone was administered for 4 weeks before mating and there were no effects on the reproductive system. There were also no effects on sperm count or motility, or on the testes, seminal vesicles, and prostate weights in males after approximately 9 weeks of dosing; however, our study indicated that morphine could affect spermatogenesis and the Sertoli cell count. These results are in contrast to our study and others on the male reproductive effects of morphine (15), in which administration of morphine to male rats for 2 weeks (30 mg/kg

twice daily) resulted in marked decreases in the number of matings, pregnancies, and implantations per litter, and an increase in pseudo-pregnancies when the animals were mated with untreated females. These findings were not associated with decreased sperm count or motility. However, seminal vesicle and prostate weights were significantly reduced in the morphine-treated males. The authors concluded that the effects of morphine on male fertility were likely secondary to the effects on accessory sex gland secretions and potentially on the formation of the copulatory plug (16-18).

In our study, we observed that the mean sperm count and motility, and also the rate of spermatogenesis, were significantly lower in the male rats who were administered morphine.

As with females, the basis for the differences between morphine and oxymorphone on male reproductive function is not entirely clear. In the previous study, however, the dose of morphine used (30 mg/kg administered subcutaneously twice daily, or 60 mg/kg/day) (15) was 30-fold higher than the ED50 level for analgesic activity (2 mg/kg for males by subcutaneous injection) (19). Lower doses were not evaluated in that study. In the present study, the dose of morphine used for the target group (5 mg/kg intraperitoneal injections, twice a day) was lower compared to the previous study.

### 5.1. Limitations

A study design that determines the influence of morphine on reducing the exact hormones (LH, FSH, or testosterone) is essential, and discovery of the mechanism of morphine's effects on the genital and hormonal systems is needed in future studies. Human studies will be necessary to identify the exact effects of morphine on the human reproductive system.

### 5.2. Conclusion

If the results of this study could be generalized to humans, with regard to morphine's properties and its increasing usage among human populations, infertile individuals with a history of drug abuse could be informed about the known short- and long-term effects on the genital system. Many of these effects, especially hyaline degenerative changes of the seminiferous tubules, are irreversible and result in permanent infertility.

### Acknowledgments

We are thankful to the research council of Mashhad University of Medical Sciences for the financial support.

## Footnotes

**Authors' Contribution:** Hasan Ahmadnia, Alireza Akhavan Rezaayat, Mahmood Hoseyni, Nooriye Sharifi, and Mollahamad Khajedalooe: Study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, study supervision, and administrative, technical, and material support. Arash Akhavan Rezaayat: Analysis and interpretation of data, drafting of the manuscript, statistical analysis, and critical revision of the manuscript for important intellectual content.

**Financial Disclosure:** We were supported financially by the research council of Mashhad University of Medical Sciences.

## References

- Mokri A. Brief overview of the status of drug abuse in Iran. *Arch Iranian Med.* 2002;**5**(3):184-90.
- Daniell HW. Hypogonadism in men consuming sustained-action oral opioids. *J Pain.* 2002;**3**(5):377-84. [PubMed: [14622741](#)].
- Teusch L, Scherbaum N, Bohme H, Bender S, Eschmann-Mehl G, Gastpar M. Different patterns of sexual dysfunctions associated with psychiatric disorders and psychopharmacological treatment. Results of an investigation by semistructured interview of schizophrenic and neurotic patients and methadone-substituted opiate addicts. *Pharmacopsychiatry.* 1995;**28**(3):84-92. doi: [10.1055/s-2007-979596](#). [PubMed: [7568370](#)].
- Cicero TJ, Wilcox CE, Bell RD, Meyer ER. Acute reductions in serum testosterone levels by narcotics in the male rat: stereospecificity, blockade by naloxone and tolerance. *J Pharmacol Exp Ther.* 1976;**198**(2):340-6. [PubMed: [948030](#)].
- Yilmaz B, Konar V, Kutlu S, Sandal S, Canpolat S, Gezen MR, et al. Influence of chronic morphine exposure on serum LH, FSH, testosterone levels, and body and testicular weights in the developing male rat. *Arch Androl.* 1999;**43**(3):189-96. [PubMed: [10624501](#)].
- Cicero TJ, Adams ML, Giordano A, Miller BT, O'Connor L, Nock B. Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. *J Pharmacol Exp Ther.* 1991;**256**(3):1086-93. [PubMed: [2005573](#)].
- Gabriel SM, Clark JT, Kalra PS, Kalra SP, Simpkins JW. Chronic morphine and testosterone treatment: effects on norepinephrine and serotonin metabolism and gonadotropin secretion in male rats. *Brain Res.* 1988;**447**(1):200-3. [PubMed: [2454704](#)].
- Vathy I. Prenatal opiate exposure: long-term CNS consequences in the stress system of the offspring. *Psychoneuroendocrinology.* 2002;**27**(1-2):273-83. [PubMed: [11750783](#)].
- Malanga CJ, Kosofsky BE. Mechanisms of action of drugs of abuse on the developing fetal brain. *Clin Perinatol.* 1999;**26**(1):17-37. [PubMed: [10214541](#)] v-vi.
- Byrnes JJ, Babb JA, Scanlan VF, Byrnes EM. Adolescent opioid exposure in female rats: transgenerational effects on morphine analgesia and anxiety-like behavior in adult offspring. *Behav Brain Res.* 2011;**218**(1):200-5. doi: [10.1016/j.bbr.2010.11.059](#). [PubMed: [21138744](#)].
- Johnson NL, Carini L, Schenk ME, Stewart M, Byrnes EM. Adolescent opiate exposure in the female rat induces subtle alterations in maternal care and transgenerational effects on play behavior. *Front Psychiatry.* 2011;**2**:29. doi: [10.3389/fpsy.2011.00029](#). [PubMed: [21713113](#)].
- Ghowsi M, Yousofvand N. Impact of morphine dependency and detoxification by methadone on male's rat reproductive system. *Iran J Reprod Med.* 2015;**13**(5):275-82. [PubMed: [26221126](#)].
- Gabriel SM, Simpkins JW, Kalra SP, Kalra PS. Chronic morphine treatment induces hypersensitivity to testosterone-negative feedback in castrated male rats. *Neuroendocrinology.* 1985;**40**(1):39-44. [PubMed: [2982110](#)].
- Cicero TJ, Meyer ER, Wiest WG, Olney JW, Bell RD. Effects of chronic morphine administration on the reproductive system of the male rat. *J Pharmacol Exp Ther.* 1975;**192**(3):542-8. [PubMed: [1168254](#)].
- Cicero TJ, Davis LA, LaRegina MC, Meyer ER, Schlegel MS. Chronic opiate exposure in the male rat adversely affects fertility. *Pharmacol Biochem Behav.* 2002;**72**(1-2):157-63. [PubMed: [11900783](#)].
- Miranda-Paiva CM, Nasello AG, Yin AJ, Felicio LF. Morphine pretreatment increases opioid inhibitory effects on maternal behavior. *Brain Res Bull.* 2001;**55**(4):501-5. [PubMed: [11543950](#)].
- Vassoler FM, Byrnes EM, Pierce RC. The impact of exposure to addictive drugs on future generations: Physiological and behavioral effects. *Neuropharmacology.* 2014;**76 Pt B**:269-75. doi: [10.1016/j.neuropharm.2013.06.016](#). [PubMed: [23810828](#)].
- Slamberova R, Szilagyi B, Vathy I. Repeated morphine administration during pregnancy attenuates maternal behavior. *Psychoneuroendocrinology.* 2001;**26**(6):565-76. [PubMed: [11403978](#)].
- Peckham EM, Traynor JR. Comparison of the antinociceptive response to morphine and morphine-like compounds in male and female Sprague-Dawley rats. *J Pharmacol Exp Ther.* 2006;**316**(3):1195-201. doi: [10.1124/jpet.105.094276](#). [PubMed: [16291875](#)].